133

STRUCTURE OF THE SPLEEN OF THE NILE TILAPIA (OREOCHROMIS NILOTICUS) : LIGHT AND ELECTRON MICROSCOPIC STUDIES

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ABSTRACT

A histological investigation of the possible structure and functions of the spleen of the Nile tilapia Oreochromis niloticus was conducted by light (LM), and transmission electron microscopy (TEM). The spleen is divisible into red and white pulp and a stroma consisting of a reticular network, a collagenous capsule, and trabeculae containing smooth muscle bundles. The parenchyma of the organ is predominantly made up of the red pulp, a system of splenic cords and sinuses. White pulp areas appear to be devoid of lymphoid follicles and consist mainly of periarteriolar lymphatic sheaths (PALS). Filtering of particulate matter from the blood occurs in the red pulp by phagocytes of the pulp cords and ellipsoids (periarterial macrophage sheaths). The ellipsoids are palestaining cuffs of macrophages and reticular cells in a framework of reticular fibres surrounding the arterial capillaries. The melano-macrophage centres (MMCs) are found throughout the parenchyma of spleen and show a close association with the vascular system, i.e. splenic ellipsoids, sinusoids of red pulp and blood sinuses. They exhibit distinct degree of development from small groups of actively phagocytic macrophages to large, totally or partially encapsulated centres, where effete phagocylic cells are filled by lipofuscin and melanin. The ultrastructural data presented here suggest various specific physiological roles for the Nile tilapia Oreochromis niloticus spleen, including hematopolesis, phagocytosis, tissue breakdown and erythrocyte catabolism. Only sparse lymphopolesis and plasmapolesis were recognized,

Key words: Spleen; Oreochromis niloticus; ultrastructure; light microscope.

INTRODUCTION

The teleost spleen is a lymphoid organ whose functions are still in question. Several investigators have previously suggested its crucial roles in the immunological defense mechanisms and

Mansoura, Vet. Med. J. (133 - 154)

H. E. S. Marei et al...

hematopoiesis of fish (Ellis et al., 1976; Elils, 1980; Secombes and Manning, 1980; Tatner et al., 1984; Lamers, 1986; Van Muiswinkel et al., 1991; Alvarez et al., 1998), but only a limited number of general studies of the ultrastructure of this organ have been reported (Bielek, 1981; Zapata, 1981, 1982; Pulsford et al. 1982; Ishizeki et al., 1984; Temmink and Bayne, 1987; Douglas et al., 1990; Guesada et al., 1990; Tanaka and Goto, 1991; Alvarez et al., 1996; Alvarez et al., 1998).

With respect to the morphological and ultrastructure peculiarities of the fish spleen, it shows remarkable variations in the distribution and ratio of red and white pulp according to species (Groman, 1982; Douglas et al., 1990; Guesada et al. 1990; Alvarez et al., 1998). The presence or lack of ellipsolds and melanomacrophage centers (MMCs) has also been described (Ellis et al., 1976; Ferguson et al., 1976; Fulop and McMillan, 1984; Fange and Nilsson, 1985; Douglas et al., 1990; Herraez and Zapata, 1991; Espenes et al., 1995; Romano et al., 1997). Other ultrastructural investigations of fish spleens have limited their focus to the reticuloendothelial system (Graf and Schluns 1979; Ferguson, 1984; Fulop and McMillan, 1984; Page and Rowley, 1984; Hunt and Rowley, 1986; Ganassin and Bols, 1999).

Regarding the suggested functional aspect of telcost spleen, it is generally considered an important site of phagocytosis of particulate matter and senile blood cells. In some species it is hemopoietically active (Catton, 1951; Fey. 1965; Haider, 1966; Weiss, 1991; Romano et al., 1993), while in others only plasmapoiesis has been observed (Zapata, 1982; Schroder et al., 1998; Petrie-Hanson and Ainsworth, 2001). Its importance in immune reactivity has been questioned. Spleneetomy in the blue gourami (Trichogaster trichopterus) prevented antibody formation (Yu et al., 1970), but had no effect on antibody response in Lutjanus griseus (Ferren, 1987).

We are currently conducting a project on tilapla fish as a biological marker for insecticide pollution particularly the possible impact of such pollution on the structure of immune organs and its cellular immune response. Since to the best of our knowledge a detailed investigation on the cytology of the Nile tilapla. **Oreochromis niloticus** spleen in general and its exact function in particular is still in need for a precise clarification. We examined the histology and ultrastructure of Oreochromis niloticus spleen so that we could more precisely elucidate its cytology and ultrastructure morphology, in order to obtain the basic cytological information needed for our project concerning the effects of insecticide pollution on the cellular immune responses in this species.

MATERIAL AND METHODS

Twenty specimens of Nile tilapia **Oreochromis niloticus** fish were captured from the Nile River, Mansoura, Egypt, in the summer of 2002. Upon capture, the fish were Immediately transported to our laboratory at faculty of Vetermary Medicine. Mansoura University where they were maintained for 14 days in a flow -through (Nile River water). 200-L aquarium. All fish were led on commercial diets and sacrificed by decapitation.

For light microscopy, the spleens were fixed by immersion in 10% neutral buffered formalin and Bouin's fluid. Paraffin sections of 6 μ m were prepared and stained with hematoxlin and cosin. Crossman trichrom, reticulin method and periodic acid-Schiff (PAS). The aforementioned methods were carried out as outlines by **Carson (1990)**.

For electron microscopy, portions of the spleens were cut into 1.0-2.0-mm pieces and placed immediately into cold (0-4°C) 2 % glutaraldehyde in 0.1 M phosphate buffer at a pl1 of 7.4 for 48 h. The tissues were washed several times [10min/wash) in the above buffer and postfixed in 1% osmium (ctroxide in 0.) M phosphate buffer (pH 7.4) for 1 h at noom temperature. The tissues were dehydrated in a graded series of ethanols and subsequently in propylene oxide. Tissue sections then were infiltrated by Epon resin and propylene oxide mixtures at a resin-propylene oxide ratio of 1:1 for 60 minutes and 3:1 for 4 h. and finally with 100% restn overhight. They were then embedded in fresh 100% resin and polymerized at 60 C. Thicker sections (1-2 mm) were eut with glass knives, stained with foluidine blue, and examined by light microscope to select ureas for ultrastructural study. Ultrathin sections (60-90 mm) were obtained with an electron microscope.

RESULTS

The spleen of the Nile tilapia **Oreochromis niloticus** was recognized as circumscribed highly eclular mass enclosed within a thick connective tissue capsule (Fig. 1). The capsule consisted of fibroblasts, collagen fibers, smooth muscle cells and was covered by a single layer of mesothelial cells (Fig. 2). Thin trabeculae projected from the capsule into the outer area of the splenic parenchyma (Fig. 3). The capsule and trabeculae are demarcated by reticular cells which were interconnected in the reticular cell network of the splenic pulp (Figs. 2, 3). The reicular cells presented cytoplasmic processes exhibiting few filamentous bundles; their eccentric, oval, euchromatinic nuclei showed marginal and central heterochromatin with a prominent nucleolus. A few mitochondria, a well-developed Golgi complex, sparse rER tubules, numerous free ribosomes, and tew spherical electron-dense granules were observed (Figs. 4, 5).

Mansoura. Vet. Med. J.

H. E. S. Marei et al...

The subcapsular region of the splenic parenchyma was composed of a network of reticular cells enclosing within their meshes a considerable number of macrophages and blood cells, no specific cell type was predominating. Except for the subcapsular region, the splenic parenchyma was composed of red and ill-developed white pulp without clear demarcation (Fig.6). The red pulp constituted most of the splenic parenchyma and was composed of an extensive interconnected system of splenic cords and sinusoids (Figs. 7, 8). The splem cords were composed of a network of reticular cells joined by desmosomes, and were tightly packed with a varying number of crythrocytes, heterophilic granulocytes, cosinophilic granulocytes, thrombocytes, and macrophages (Fig.9).

The heterophilic granulocytes are oval with an eccentric oval non-indented nucleus showing peripheral and central clumps of heterochromatin. The cytoplasm contains a small Golgi apparatus, sparse eisternae of rER, free ribosomes, and large mitochondria. Numerous vesicles and oval or spherical granules that vary in size and density were observed. These granules were homogeneously dense (Fig. 9). Other immature forms of heterophilic granulocytes found in the spleen were heterophilic myelocytes with their dense cytoplasm and characteristically striated granules, and promyelocytes with early signs of granule formation.

Eosinophilic granulocytes were oceasionally seen in the splenic parenchyma. Their lobulated euchromatic nuclei exhibited central and peripheral heterochromatin. The cytoplasm contains a well-developed Golgi complex, eisternae of rER and sER, few mitochondria, and oval or spherical granules of varying size and density.

Different regions of the red pulp contained varying numbers of ervthrocytes or their precursors in isolation or in groups where they were irregularly contoured (Figs. 9, 10).

The thrombocytes were characterized by a central or eccentric nucleus and a marginal band of microtubules and vesiculated r ER (Figs. 10, 11). Sparse mitochodria, small Golgi complex, numerous free ribosomes, and a little rER were found as well (Fig. 11).

Splenic sinusoids were large irregular channels lined by endothelial cells and fenestrated basal lamina (Fig. 12). The endothelial cells were flattened with irregular cuchromatic nuclei shawing marginal and central heterochromatin. They lack cell junctions and their cytoplasm contains a well-developed juxtanuclear Golgi apparatus, sparse rER tubules, numerous free ribosomes, mitochondria, oval or spherical electron dense lysosomes-like granules, and numerous microplnocytotic vesicles on the luminal and basal surface.

Prominently leatured within the splenic red pulp were numerous macrophages. They were irregularly shaped cells with eccentric eucliromatic nucleus with peripheral chromatin and a distinct nucleolus (Fig. 13). Their cytoplasm contained a distinct Golgi apparatus, some mitochndria with few cristae, sparse r ER tubules, free ribosomes, and many lysosomes and phagolysosomes which often contained phagocytosed erythrocytes, lymphocytes (Fig. 14) or large, pigmentbearing inclusion bodies (Figs. 13).

MMCs are aggregations of closely packed macrophages (Figs. 15, 16), the limits of which were difficult to discern. They were found throughout the parenchyma of spleen and showed a close association with the vascular system, i.e. splenic ellipsoids, sinusoids of red pulp and blood sinuses (Fig. 17). They exhibited distinct degree of development from small groups of actively phagocytic macrophages to large, totally or partially encapsulated centres, where effect phagocytic cells were filled by cell debris. An interrupted capsule of collagen fibers and reticular cells enclosed them. Lymphoid cells were commonly found near the MMCs.

The white pulp surrounds the arterial blood vessels and MMCs or forms small clusters of lymphoid cells, heterophilic granulocytes, thromboeytes, and macrophages, all within a thin framework of dense reticular cells (Fig. 18). Plasma cells were very sparse, while lymphocytes were only infrequently recognized. Reticular cells of the white pulp were stellate with long processes joined by desmosomes. The nuclei were irregular, euchromatic with marginal and central heterochromatin, and a nucleolus. The electron-dense cytoplasm contained, in addition to the usual organelles, numerous bundles of filaments and sparse electron-dense granules. The macrophages of the white pulp have a more heterogeneous appearance than those in the red pulp. They contained lysosomes of different sizes and homogeneous, granular, or filamentous content: sometimes melanin granules, multivesicular bodies, and involuted crythrocytes and lymphocytes were found.

Lymphocytes were characterized by their sparse cytoplam and spherical nucleus with great amounts of central and marginal heterochromatin. Lymphoblasts were larger cells with an oval nucleus with little heterochromatin and a prominent nucleolus and more abundant cytoplasm (Fig. 18).

The main arterial supply of the spleen come from the splenic artery which enters through the hilus and divide at the organ center, forming two main branches. Numerous radially arranged arterioles originate from the main branches and were finally continued as capillaries. The arterial branches were accompanied by a wide lunen. The capillaries were frequently surrounded by periarterial macrophage sheaths or ellipsoids. These sheathed capillaries consisted of an endothelium that was not limited by a basal lamina and were surrounded by a network of collagen bundles, reticular fibers within whose wide meshes, macrophages, and blood cells were distributed (Fig. 17, 18). The periarterial macrophages frequently contained phagocytosed crythrocytes. It was difficult to ascertain whether the capillaries open directly into the spaces among the retic-

H, E, S. Marei et al...

ular eells of the splenic cords or communicate directly into the human of the sinusoids.

DISCUSSION

The exact functional roles of the feleost spleen are up till now still a matter of debate. Several functions have been attributed to it, most of them are poorly understood. It is a hemopoietic organ which may be phagocytic, a store of crythrocytes, and a site of plasma cells development and consignently antibody production.

The present study demonstrated that the splenic parenchyma of the Nile tilapia **Oreochromis niloticus** consists of red pulp, whereas the white pulp is generally poorly developed, as in **Lepomis sp. (Fulop and McMillan, 1984)**, **Salmo gairdneri**, **Pleuronectes platessa**, **Cyprinus carplo (Lamers, 1985)**, and stripped bass **Morone saxatilis (Douglas et al., 1990)**. In the icefish, however, lymphoid cells and marcophages are the dominant cells of a splcen parenchyma which show practically no erythrocytes **(Walvig, 1985)**. The different functional capacities among the teleost splcen can be related to the ratio of red and white pulp.

Beside the poorly developed white pulp that was clarified here, our results on the Nile tilapia Oreochromis niloticus spleen revealed the presence of certain unique structural features. Similar to the findings of **Douglas et al (1990)**, we could not identify in our material the developmental stages of plasma cells. These stages were reported to be numerous by Zapata (1982) in reach Rutilus rutilus and gudgeon Gobio gobio. Moreover, in the present study fewer lymphoeytes (or lymphoblasts) were observed with the absence in our material of lymphocyte clusters surrounding monocytes or macrophages. An arrangement of lymphoid cells and macrophages has been described in the spleen of roach Rutilus rutilus (Zapata, 1982) and of the doglish Scylioorhinus canicula (Pulsford et al., 1982). In agreement with our findings, the spleen in the Oriental weatherlish (also known as loach) Misgurnus anguillicaudatus showed close proximity of the constituent cells to each other and no specific orientation of the lymphocytes to monocytes or macrophages (Ishizeki et al., 1984). Unique to the Oriental weatherfish, however, was that the principal cell (ypc in the spleen was cosinophils (mature and immature stages) whose granules have a crystalline core (Ishizeki et al., 1984). In the present study, cosinophils were infrequently encountered, as has been demonstrated previously (Bodammer, 1986). Moreover, a predominant feature of the Nile tilapia Oreochromis niloticus spleen was the presence of a considerable number of crythrocytes and crythroblasts as well as numerous thrombocytes in various stages of development, suggesting that both cell types may have their origin in this fissue.

Macrophages, a prominent feature in the spleens of Nile filapia **Oreochromis niloticus**, liequently contained recognizable red cells or presumed fragments thereof within their phago-

somes. This observation is consistent with the general role of these cells in erythrocytes destruction in vertebrates, and has been confirmed ultrastructurally for other fish species such as dogfish (Pulsford et al., 1982) and sunfish Leponis spp. by Fulop and McMillan (1984).

Histological analysis of Nile tilapia **Oreochromis nilotieus** spleen demonstrated closely packed phagocytic cells containing abundant pigment. These pigmented macrophage aggregates were similar to those described for sunfish (**Fulop and McMillan, 1984**): they were surrounded by lymphoid cells, a morphological relationship that may facilitate their proposed role in antigen processing (**Aglus 1981, 1985**). In the present study, no evidence for the uptake of cell products has been observed in the macrophages of MMCs. These findings were in harmony to those of **Fulop and McMillan (1984**) in Leponits sp. and might suggest that such macrophages have no active role in the process of cellular degradation. In contrast to our findings, **Quesada et al. (1990**) in the sea hass demonstrated the presence of fragments of erythrocytes, lysosomes, inclanin granules, and residual bodies within the cytoplasm of MMCc macrophages suggesting the involvement of theses cells in erythrocyte degradation. Herraez and Zapata, 1991 reported that the main inclusion observed in the MMCs of **Carassius auratus** is lipofusein with little baemosiderin and suggest various non-specific physiological roles for the teleost MMCs, including tissue breakdown and crythrocyte catabolism.

White pulp in Nile tilapia **Oreochromis niloticus** is formed of reticular fibers intermingled with lymphoid cells, macrophages, granulocytes, thrombocytes, and some isolated crythrocytes. White pulp is sparse and forms a cuff around the pulp arteries and MMCs and appears diffusely in the splenic parenchyma as in some other species (**Pitchappan**, 1980; **Groman**, 1982; **Fulop and McMillan**, 1984; **F nge and Nilsson**, 1985). Moreover, the spleen of Nile tilapia **Oreochromis niloticus** shows small groups of lymphocytes and plasma cells between the splenic cords, as in the fresh-water teleosts **Rutilus rutilus** and **Gobio gobio (Zapata**, 1982). In contrast to our findings. **Tomonaga et al. (1992)** demonstrated that the major cellular constituent of the splenic white pulp of the Aleutian skate (**Bathyraja aleutica**) was plasma cells with only a small number of lymphocytes. These findings indicate that in contrast to the Nile tilapia **Oreochromis niloticus** white pulps, the splenic white pulp is the major site for immunoglobulin production in this fish. **Petric-Hanson and Ainsworth (2001)** demonstrated the presense of immunoglobulin positive plasma cells were first detected on day 14 post-hatching in the spleen of channel catfish and their was a constant increase in their mumber with icreasing age.

The arterial supply of the Nile tilapia **Oreochromis niloticus** spleen comprised a longitudinal artery and vein lying side by side, along the length of the spleen. The artery gives off radial branches toward the splenic pulp. The arterioles terminate as capillaries in the red pulp. Frequently the capillaries are surrounded by an ellipsoid, as in most teleosts (**Graf and Schluns**,

Mansoura, Vet. Med. J.

1979; Fulop and McMillan, 1984; Lamers and De haas, 1985). The function of the ellipsoid is poorly understood. The filaments and numerous micropinocytotic vesicles of the endothelial cells of the ellipsoid in sea bass can be related to the regulation of capillary diameter and transport across endothelial cells (Quesada et al., 1990). Espenes et al., 1997 demonstrated the importance of ellipsoidal macrophages in the clearance of filtered substances trapped in the reticular matrix of the ellipsoidal wall. The presence of erythrocytes in varying degrees of degradation in the perlarterial macrophages that has been revealed in the present study might suggest an active role of ellipsoid in the process of erythrocyte destruction. In contrast to our findings, a lack of cellular breakdown in the ellipsoids was noted in the sumfish (Fulop and McMillan, 1984). Espenes et al., 1995 suggests a specific role for the splenic ellipsoids in rapid immune-complex trapping and invites speculation on its significance in a secondary immune response.

As in other species, (Lepomis sp, Salmo gairdneri, Leuciscus idus), it is difficult to ascertain whether the splenic capillaries in the Nile tilapia **Oreochromis niloticus** open into the reticular network or are continuous with the sinusoids.

From the present study, phagocytosis, hemopoiesis and crythrocytes degradation seems to be the main functions of Nile tilapía **Oreochromis niloticus** spleen. Only sparse lymphopoiesis and plasmapoiesis are found, the latter being described in the fresh water teleosts **Rutilus rutilus** and **Gobio gobio (Zapata, 1982)** and chaneel catfish (**Petrie-Hanson and Ainsworth, 2000**). The role of teleost spleen in immune reactivity has been questioned. The activity of antibody production in the spleen could be indicated by the numerous plasma cells it contains. Splenectomy had no effect on antibody response in **Lutjanus griseus (Ferren, 1967)** but abolish it in **Trichogaster triebopterus (Yu et al., 1970**). In frilled shark, **Chlamydoselachus anguineus**, no active hemopoiesis is noted and the major splenic function seemed to be restricted to phagocytosis of worn out red blood cells **(Tanaka and Goto, 1991**).



Fig. 1 : Photomicrograph of a section of the **Oreochromis niloticus** spleen. Note circumscribed highly cellular mass enclosed within a thick connective tissue capsule. PAS X 40.



Fig. 2 : Photomerograph of a section of the Orcochroneis miloticus spicen. The capsule consistent of fibrobiasts, collagen libers, smooth nuisele cells. Masson's tricknome X 1000.

Mansoura, Vet. Med. J.



Fig. 3 : Photomicrograph of a section of the Oreochromis niloticus spleen. Note small trabeculae (T) projected from the capsule and the reticular cells (R) that demarcate the inner surface of the trabeculae. Masson's trichrome X 1000.



Fig. 4 : Electron micrograph of Oreochromis niloticus splenic reticular cells. Note the nucleus (N), cytoplasmic process, mitochondria (arrows), Golgi apparatus, rER tubules (E) X 10000.

Mansoura, Vet. Med. J.

Vol. V, No. 1, 2003

142



Fig. 5 : Higher magnification of figure (4) showing the nucleus (N), cytoplasmic process (C), and rER tubules (E) X 22000.



Fig. 6 : Photomicrograph of a section of the Oreochromis niloticus spheen. The spheric parenchyma is composed of red and ill-developed white pulp without clear demarcation. PAS X 1000.



Fig. 7: Photomicrograph of a section of the **Oreochromis niloticus** spleen. The red pulp constituted most of the splenic parenchyma and was composed of an extensive interconnected system of splenic cords (C) and sinusoids (S). PAS X 1000.



Fig. 8 : Photomicrograph of a section of the Oreochromis niloticus spleen. Note the sinuses (S) and splenic cords (C). Masson's trichrome X 1000.

Mansoura, Vet. Med. J.



Fig. 9: Electron micrograph of Oreochromis niloticus splenic red pulp. Note reticular cells (R), erythrocytes (E), heterophilic granulocytes (E), thrombocytes (T), and macrophages (M) X 4600.



Fig. 10 : Electron micrograph of Oreochromis niloticus spienie (ed pulp. Note reticular cells (R), crythrocytes (E), thrombocytes (T) X 6000.



Fig. 11 : Higher magnification of thrombocytes (T). Note nucleus (n), a marginal band of microtubules (T) and vestculated r ER (arrow) X 8000.



Fig. 12 : Photomicrograph of a section of the Oreochromis niloticus spleen. Note splenic sinusoids (S) as a large irregular channels lined by endothelial cells (E) and a fenestrated basal lamina (arrow). PAS X 1000.

Mansoura, Vet. Med. J.



Fig. 13 : Electron micrograph of Oreochromis nilotieus splenic red pulp. Note macrophage with cecentric indented nucleus (N), mitochondria (M) and phagosomes (P) X 10 000.



Fig. 14 : Electron micrograph of Oreochromis niloticus splenic red pulp. Note phagocytosed lymphocyte (L) inside the cytoplasm of the macrophage (M). X 8 000.

Mansoura, Vet. Med. J.



Fig. 15 : Electron micrograph of Oreochromis niloticus splenic red pulp. Note the melanomacrophage (MM) X 8 000.



Fig. 16 : Higher magnification for the melano-macrophage in the splenic red pulp. Note the nucleus (N), melanin granules (G) inside its cytoplasm X 13 000.

Mansoura, Vet. Med. J.



Fig. 17 : Photomicrograph of a section of the Oreochromis niloticus spleen. Note the close proximity of melano-macrophages (arrow) to the splenic sinusoids (S). Reticulin X 1000.



Fig. 18: Photomicrograph of a section of the Oreochronnis mioticus sphere. Note sheathed capillary (ellipsoid) (E) and macrophages (M), all within a thin framework of dense reticular cells (R). Masson's trichcome X 1000.

Mansoura, Vet. Med. J.

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الملخص العربى تركيب الطحال فى أسماك البلطى النيلى : دراسات بواسطة الميكروسكوب الضوئى والإلكترونى

تم دراسة التركيب والوظيفة المحتملة لطحال أسماك البلطي النيلي وذلك باستخدام كل من الميكروسكوب الضوئي والإلكتروني، غيرَ طحال أسماك البلطي النيلي بانقسامه إلى اللب الأحمر red pulp واللب الأبيض white pulp بالإضافة إلى المحفظة الخارجية والتي تكونت من ألياف كولاجينية وشبكية بالإضافة للخلايا العضلية الملساء إحتل اللب الأحمر معظم مناط الطحال وتكون أساساً بنظام متشابك من الأحبال والجيوب الطحالية، أما اللب الأبيض فلقد تميز بغياب الحويصلات الليمفاوية المميزة للطحال والمتواجدة في طحال الحيوانات الثديبة حيث تكون من مجموعة من الأغلفة الليمغاوية المتركزة حول بعض الشرايين الصغيرة، ومن خلال التعرف على التركيب الدقيق فلقد تم الإشارة إلى بعض الوظائف الأساسية لطحال الأسماك وخاصة دورة في ترشيح الأجسام الغزيبة والصلبة من الذم وذلك عن طريق الخلايا البلعمية المتواجدة في مناطق اللب الأحمر وفي مناطق الـ ellipsoids حيث ظهرت تلك المناطق كمناطق خافتة الصبغة مكونة أساساً من خلايا بلعمية وخلايا وأليات شبكية في بعض المناطق المحيطة بالشعيرات الدموية، كما قيز الطحال باحتوائية على العديد من الخلايا البلعمية الصبغية حيث إنتشرت تلك الخلايا خلال جميع مناطق الطحال حيث تركزت تلك الخلايا بوجودها بالقرب من الأوعية الدموية، ولقد أظهرت تلك الخلايا تفاوت واضع في درجات وضوحها وتطورها، ومن هذه الدراسة تم إستئتاج الدور الوظيفي المحتمل لطحال الأسماك حيث أظهرت تلك الدراسة قيام الطحال بدور حيوي في عمليات تخليق خلايا الدم المختلفة وكذلك دورة الرئيسي في التخلص من كرات الدم الحمراء المتهالكة، ومقارنة بوظائف الطحال في أنواع الأسماك، والثدييات الأخرى فلقد تميز طحال أسماك البلطي النيلي بعدم قيامه بدور رئيسي في عمليات تخليق الخلايا المصلية والخلايا الليمفاوية مما قد يشير إلى عدم قيام طحال تلك الأسماك بدور حيوي في عمليات الاستجابة المناعية المتخصصة.

Vol. V, No. 1, 2003